Prevalence and Drug Resistance Pattern of *Staphylococcus aureus* Clinical Isolates in Bastar Region

Bastar Bölgesi'nde *Staphylococcus aureus* Klinik İzolatlarının Yaygınlığı ve İlaç Direnci

İlaca Dirençli *S. aureus* Yaygınlığı / Drug Resistant

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Özet

Amaç: Bu araştırmanın amacı Bastar bölgedeki yaygınlığını ve *S. aureus* antibiyotik direncini etüd edilerek. Gereç ve Yöntem: Ocak 2010-Mayıs 2012 tarihinden itibaren, 916 *S. aureus* izolatı kültüre klinik örneklerden standart testlerle tespit edilmiştir. MRSA tespit oksasilin ekran ağar tarafından yapılan ve sefoksitin disk difüzyon testleri yapıldı. Kirby-Bauer disk difüzyon testi antibiyotik duyarlılık incelemek için kullanılmıştır. Vankomisin MIC ve linezolid Etest tarafından incelendi. Bulgular: Izolatların % 34.8 MRSA idi. MRSA izolatları içinde,% 63 (165) eritromisine dirençli bulunmuştur, azitromisine % 39.3 (103), klindamisin için % 61.8 (162), % 81.5 (260) kotrimoksazol için, % 0.6 (2) linezolide, % 0.9 (3) vankomisine, % 76.5 (244) tetrasiklin, % 67.7 (216) gentamisin için % 63.3 (202) siprofloksasin, % 8.5 (27) gatifloksasin için, % 16.4 (43), kloramfenikol % 68.4 (39) norfloksasin nitrofurantoine % 12.3 (7), Sulfısoxazol için % 80.7 (46) ve % 80.7 (46) antibiyotik trimetoprim için. Tartışma: Çalışması bu bölgeden ilk kez yapıldı. Prevalans ve ilaç direnci oranı diğer çalışmalar ile karşılaştırılmıştır. Linezolid direncinin ortaya çıkması ve MRSA izolatlarının görece yüksek vankomisin direnci bu çalışmanın endişe verici bir bulgudur. Kotrimoksazol ve/veya gentamisin ilk ampirik tedavisi olarak kabul edilebilir, ancak Antibiyograma göre doğru bir antibiyotik ile hemen değiştirilmelidir.

Anahtar Kelimeler

Bastar; MRSA; Prevalans; İlaç Direnci

Abstract

Aim: The aim of this research was to study the prevalence and antibiotic resistance of *S. aureus* in the Bastar region. Material and Method: From the clinical samples cultured from Jan 2010 to May 2012, 916 *S. aureus* isolates were identified by the standard tests. Screening of MRSA was done by oxacillin screen agar and cefoxitin disk diffusion tests. Antibiotic susceptibility was examined by Kirby-Bauer disk diffusion test. For MIC of vancomycin and linezolid, Etest was performed. Result: Of the isolates, 34.8% were MRSA. In the MRSA isolates, 63% (165) were found resistant to erythromycin, 39.3% (103) to azithromycin, 61.8% (162) to clindamycin, 81.5% (260) to cotrimoxazole, 0.6% (2) to linezolid, 0.9% (3) to vancomycin, 76.5% (244) to tetracycline, 67.7% (216) to gentamycin, 63.3% (202) to ciprofloxacin, 8.5% (27) to gatifloxacin, 16.4% (43) to chloramphenicol, 68.4% (39) to norfloxacin, 12.3% (7) to nitrofurantoin, 80.7% (46) to sulfisoxazole, and 80.7% (46) to trimethoprim antibiotics. Discussion: The study was conducted first time from this region. The prevalence and drug resistance percentage is compared with other studies. Emergence of linezolid resistance and relatively higher vancomycin resistance in the MRSA isolates is a worrisome finding of this study. Cotrimoxazole and/or gentamycin may be considered as initial empiric treatment, but must be replaced immediately with the correct antibiotic according to the antibiogram.

Keywords

Bastar; MRSA; Prevalence; Drug Resistance

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Introduction

Staphylococcus aureus is a well known nosocomial pathogen and the methicillin resistance in the organism has been more than a half century old issue [1–4]. Methicillin resistant *S. aureus* (MRSA) isolates not only possess intrinsic ability to resist the standard concentrations of ß-laclam antibiotics and cephalosporins, but also show multiple drug resistance (MDR) to various other antimicrobial classes. MDR leaves a limited choice for antimicrobial therapy and the infections become difficult-to-treat that in turn have a significant impact in increasing morbidity, mortality, and hospital cost [5–7].

Increasing MRSA prevalence is a global problem, and regional periodic survey for antimicrobial susceptibility is a necessary step for understanding the correct empiric therapy and to develop antimicrobial stewardship to help stop the further emergence of drug resistance. A recent multicenter study including various national regions found 41% MRSA prevalence in India with the maximum prevalence being 60-68% from tertiary care centers in Central, South, and East India [8]. A very low prevalence of 2.4% was reported in 1996 from Vellore [9]. The prevalence has also been shown to be 54-57% reported from the hospitals in West, South, and North India [8, 10–12].

The tribal area Bastar is located in the central part of India as a district of Chattisgarh state. The region is a beautiful dense forest area and a well known red zone for Naxalite (Maoist) activity. Bastar natives are mostly tribes living in a primitive style under below poverty line [13, 14]. Though pyogenic wound and soft tissue infections are very common in the tribal patients attending the hospital, but there is a lack of published literature on the prevalence of pathogens and their pattern of drug resistance from this region. The aim of this research was to study the prevalence of *S. aureus* infections in the Bastar tribes and the drug resistance pattern in the isolates with an intension to help start the appropriate empirical antibiotic treatment of patients even on the levels of primary healthcare centers.

Material and Method

Study population

The study was conducted in the microbiology laboratory of School of Life Sciences at MATS University, Raipur, Chattisgarh, India. The study included the samples of patients of Bastar region attending Maharani Hospital, Jagdalpur, Bastar. The patients were mostly the tribals and belonging to the in and around area of Jagdalpur under Bastar district only. Permission for sample collection was taken from the Superintendent of the hospital. Informed consent was taken from the patients or their relatives to take samples for microbiological investigations. The institutional ethical committee approved the study protocol. Those samples which were drawn for the microbiological investigations under hospital were also included.

From January 2010 to May 2012, a total 916 *S. aureus* isolates were cultured from the clinical samples of 573 male, and 343 female patients. According to their ages, the subjects in the studied population were observed into 3 groups: (1) up to 13 yrs age, (2) 14 to 40 yrs age, and (3) >40 yrs age. The studied male population was comprised of 71 subjects of up to 13 yrs age, 347 subjects of 14-40 yrs age, and 155 subjects with >40 yrs age. In the studied female population, there were 72 subjects of up to 13 yrs age, 218 subjects with 14-40 yrs age, and 53 subjects of >40 yrs age (Table 1).

Table 1. Studied population satisfying the total study load of *S. aureus* isolates (n = 916).

Age groups	Study population	Male	Female
Up to 13 yrs	143	71	72
14-40 yrs	565	347	218
>40 yrs	208	155	53
Total	916	573	343

Culture and identification of the organisms

All the dehydrated culture media were purchased from HiMedia Lab, and prepared under instructions of the manufacturer. The pus and wound samples were taken with sterile swab sticks (Hi-Media Lab). The swabbed samples were inoculated on to sterile nutrient agar, blood agar and MacConkey agar media in Petri dishes. For throat swab, two swabs were taken from the same areas; one to prepare a smear for Gram staining, while the other to inoculate onto blood agar, chocolate agar and MacConkey agar plates. Freshly voided midstream urine samples were obtained in sterile wide-mouth screw capped universal containers. The urine specimens were well mixed and inoculated onto blood agar, MacConkey agar and cysteine lactose electrolyte deficient (CLED) agar plates. The plates were incubated aerobically at 37°C for 24 hours.

For blood culture, 3 ml blood sample was aseptically transferred to 50 ml brain-heart infusion (BHI) broth in bottles. Covering the bottle-caps with aluminiun foil, the bottles were incubated at 37 ºC. The bottles were routinely inspected twice a day (at least for the first 3 days) for signs of microbial growth for the maximum of 7 days. The aspirated broth was examined microscopically by Gram-staining, and sub-cultured aseptically onto sterile blood agar, MacConkey agar, and mannitol salt agar plates. The blood cultures were considered negative, where there were no growth occurred after 7 days.

The isolates of *S. aureus* were identified based on standard tests (Gram staining, catalase, and coagulase). The identification was further confirmed by culturing the organism on mannitol salt agar and by Dry Spot Staphytect Plus® (Oxoid); latex agglutination assay kit for *S. aureus*. The study excluded Gram negative bacilli, streptococci, and coagulase negative staphylococci (CoNS) cultured from the clinical samples.

MRSA screening

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. Plates were inoculated with 10 µL of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35°C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant [15, 16].

For further confirmation, all the isolates were subject to cefoxitin disk diffusion test using a 30 µg disk. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture was done on MHA plate. Plates were incubated at 35°C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 21 mm was reported as oxacillin resistant and ≥ 22 mm was considered as oxacillin sensitive [15, 17–19].

Kirby-Bauer disk diffusion method for antibiotic susceptibility

The antibiotic susceptibility pattern of all isolates was determined by Kirby Bauer disk diffusion method against the following antibiotics (HiMedia Laboratories Pvt. Ltd, India): penicillin-G (10 U), ampicillin (10 µg), ampicillin/sulbactam (10/10 µg), erythromycin (15 µg), azithromycin (15 µg) , clindamycin (2 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), linezolid (30 µg), vancomycin (30 µg), tetracycline (30 µg), gentamycin (10 µg), ciprofloxacin (5 µg), gatifloxacin (5 µg), and chloramphenicol (30 µg). For the isolates cultured from urine samples, norfloxacin (10 µg), nitrofurantoin (300 µg), sulfisoxazole (300 µg) and trimethoprim (5 µg) were tested in addition to the above mentioned antibiotics except erythromycin, azithromycin, clindamycin and chloramphenicol. All the tests were performed on Müeller Hinton agar, and were interpreted after incubation for 24 h at 37°C. Following CLSI criteria, the susceptibility was noted as per the zone diameter measured around each disk [15].

Etest method for MIC

Minimum inhibitory concentration (MIC) of vancomycin and linezolid was determined using Etest (AB Biodisk, Solna, Sweden) as per manufacturer's instructions [20, 21]. Following CLSI, 2010 breakpoints [15], the susceptibility was noted.

Statistical analysis

The values were represented in the relative frequencies. The data were entered in the Microsoft Excel 2007 and analyzed statistically using chi-squared test and student t test to see the significance at 0.001 level.

Results

A total number of 3591 clinical samples were processed which included 2074 pus samples, 1130 urine samples, 260 blood samples, 116 wound swabs, and 11 throat swabs. Of the entire number of clinical samples processed, only 2520 samples were found positive for bacterial growth. The prevalence of *Staphylococcus aureus* in the culture positive samples was found 36.3% (916). The other organisms cultured were: 6.2% (155) coagulase negative staphylococci, 6.7% (169) streptococci, and 50.8% (1280) Gram negative bacilli. The incidence of *S. aureus* in the types of clinical samples is shown in Table 2.

The prevalence of MRSA and MSSA in the clinical samples has been shown in Table 3. The prevalence of MRSA was found to be 34.8% (319) in the entire studied population. In the male subjects, the prevalence of MRSA was 34.4% (197), and in the female subjects 35.6% (122). In the MRSA infected males, there were 9.6% (19) children, 58.4% (115) subjects of 14-40 yrs age, and 32% (63) subjects of >40 yrs age. The MRSA infected females included 13.1% (16) children, 65.6% (80) subjects of 14-40 yrs age, and 21.3% (26) subjects of >40 yrs age.

Table 2. Prevalence of *S. aureus* in the culture positive clinical samples.

Sample	+ samples (n)	Organisms identified			
		S. aureus	CoNS	Streptococci	GNB
Pus	1343	631 (47.0%)	118 (8.8%)	63 (4.7%)	531 (39.5%)
Urine	946	144 (15.2%)	8 (0.8%)	90 (9.5%)	704 (74.4)
Blood	116	65 (56.0%)	14 (12.1%)	0 (0%)	37 (31.9%)
Wound	104	71 (71.2%)	13 (9.6%)	12 (11.5%)	8 (7.7%)
Throat-swab	11	5 (45.4%)	2 (18.2)	4 (36.4)	0 (0%)
Total	2520	916 (36.3%)	155 (6.2%)	169 (6.7%)	1280 (50.8%)

+ samples = growth positive samples, CoNS = coagulase negative staphylococci, GNB = gram negative bacilli

Table 3. Prevalence of MRSA and MSSA in the clinical samples.

	MRSA	MSSA
Total clinical samples (n = 916)	319 (34.8%)	597 (65.2%)
Pus (n = 631)	215 (34.1%)	416 (65.9%)
Urine (n = 144)	57 (39.6%)	87 (60.4%)
Blood (n = 65)	18 (27.7%)	47 (72.3%)
Wound (n = 71)	29 (40.8%)	42 (59.2%)
Throat swab (n = 5)	0 (0.0%)	5 (100.0%)

The incidences of MRSA in pus samples (631), urine samples (144), blood samples (65), wound swabs (71), and throat swabs (5) were found to be 34.1% (215), 39.6% (57), 27.7% (18), 40.8% (29), and 0% (0) respectively (Table 3).

Figure 1 represents the antibiotic resistance pattern in the isolates of MRSA. All the MRSA isolates were found resistant to oxacillin, penicillin, ampicillin, and ampicillin-sulbactam. In the MRSA isolates, 63% (165) were found resistant to erythromycin, 39.3% (103) to azithromycin, 61.8% (162) to clindamycin, 81.5% (260) to cotrimoxazole, 0.6% (2) to linezolid, 0.9% (3) to vancomycin, 76.5% (244) to tetracycline, 67.7% (216) to gentamycin, 63.3% (202) to ciprofloxacin, 8.5% (27) to gatifloxacin, 16.4% (43) to chloramphenicol, 68.4% (39) to norfloxacin, 12.3% (7) to nitrofurantoin, 80.7% (46) to sulfisoxazole, and 80.7% (46) to trimethoprim antibiotics.

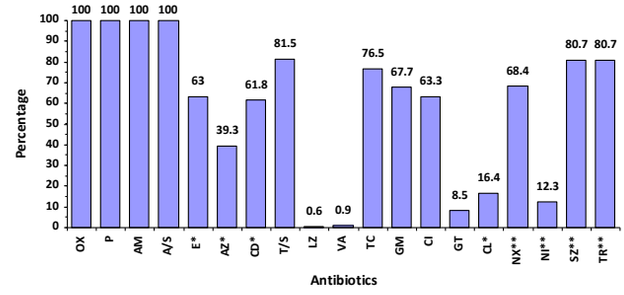

Figure 1. Percentage of MRSA isolates resistant to the selected panel of antibiotics.
* = Antibiotic used only on non-urine isolates, ** = Antibiotic used only on the urine isolates
Without asterisk = Antibiotic used for all the isolates.
OX = Oxacillin, P = Penicillin, AM = Ampicillin, A/S = Ampicillin-sulbactam, E = Erythromycin, AZ = Azithromycin, CD = Clindamycin, T/S = Cotrimoxazole, LZ = Linezolid, VA = Vancomycin, TC = Tetracycline, GM = Gentamycin, CI = Ciprofloxacin, GT = Gatifloxacin, CL = Chloramphenicol, NX = Norfloxacin, NI = Nitrofurantoin, SZ = Sulfisoxazole, TR = Trimethoprim

Discussion

S. aureus is a leading pathogen in hospital acquired infections (HAIs). The prevalence of *S. aureus* infections was next to the Gram negative bacterial infections, but on the top of Gram positive bacterial infections. However, as the isolated Gram negative bacterial pathogens were not identified to their genera or species level, the *S. aureus* infections may be considered the top leading among all the infections in the observed Bastar population. All the studied subjects were tribal and native of Bastar region only, and pyogenic and urogenital infections were found common in them. Unhygienic mode of living and least health awareness might be a cause of ease in acquiring infections.

The prevalence of MRSA varies between geographical regions and between tertiary care centers. Tertiary care centers in Central, South and East India have studied a very high MRSA prevalence of 60-68% [8]. High prevalence of 54-57% has also been shown from hospitals in West, South, and North India [8, 10–12]. Multicenter studies have revealed moderate prevalence of MRSA in India [8, 22, 23]. A recent study by Joshi et al. [8] has shown 41% MRSA prevalence in India. In mono-center studies from Karnataka, and Assam, a moderate prevalence of 35% has been observed [24, 25]. The present research has found a moderate prevalence of MRSA similar to the findings of studies by Nishi et al. [25] and Saikia et al. [24]. The prevalence is also similar to that of a multicenter study published by Mehta et al. [22].

The study recovered large number of MRSA from pyogenic infections. The most common drug in the present research to which a high percentage of MRSA isolates were resistant was trimethoprim/sulfamethoxazole (cotrimoxazole). This finding is similar to most of the earlier studies [10, 12, 23–27]. The MRSA isolates were found 61-68% resistant to gentamycin, ciprofloxacin, erythromycin, and clindamycin. The gentamycin resistance was found somewhat similar to the findings of studies by Murugan et al. [26] and Rajaduraipandi et al. [23]. A slightly lower gentamycin resistance percentage (59%) was shown by Joshi et al. [8] and Kumar et al. [27]. The MRSA isolates were shown 72-73% resistant to gentamycin in separate studies of tertiary care centres from Amritsar and Anantapur in year 2010 [10, 28]. Very high gentamycin resistance percentage of 86-90% has also been observed in some studies [12, 24, 28]. In the present study, the percentage of ciprofloxacin resistance in the MRSA isolates was found 63.3% which is nearly similar to the finding in a monocentric study in Amritsar [28]. Studies have reported lower rates of 40-48% [10, 23, 26], as well as high rates of 79-88% for ciprofloxacin [8, 12, 24, 27, 29]. Gatifloxacin is a fourth generation fluoroquinolone. Rao et al. [29] revealed 71.1% MRSA resistant to gatifloxacin. A remarkably low percentage of gatifloxacin resistance (8.5%) was found in the studied MRSA isolates.

Tertiary care centres from North-west and South India have reported 60-62% erythromycin resistance in their MRSA isolates which is quite similar to the present research [23, 26, 28]. Hospitals in other parts of our nation has increasingly reported not only 71% [8, 10], but also 80-83% erythromycin resistance in the methicillin resistant isolates of *S. aureus* [10, 12, 24, 27]. A very high erythromycin resistance percentage of 95.6% has been quoted by Rao et al. [29]. Though there is a lack of investigation of susceptibility of azithromycin in the published research articles, but Rao et al. [29] has also quoted an amazingly very high azithromycin percentage of 95.6%. The present research has found a low percentage (39.3%) of MRSA resistant to azithromycin. A recent multicentre study across India found 46.6% clindamycin resistance in MRSA [8]. Other studies reported 22% clindamycin resistance in 2012 from Pondicherry [27], 56.2% in 2009 from Assam [24]. We have observed rather higher percentage (61.8%) of clindamycin resistance in MRSA.

Vancomycin is the potential glycopeptides drug that can reliably treat MRSA infections [30]. However, the massive use of vancomycin for treating MRSA has caused the emergence of vancomycin resistant *S. aureus* (VRSA) and vancomycin intermediate *S. aureus* (VISA) cases [31]. The therapeutic and life-saving option for VRSA and VISA infections remains linezolid, first antimicrobial of oxazolidinone group available since 2000 [30]. The first case of linezolid-resistant staphylococci appeared within 1 year after linezolid was approved for therapeutic use [32]. Although linezolid resistance in *S. aureus* is uncommon, emergence has been shown from some parts of the world [33]. From India, first case report of linezolid resistance was published in 2011 from Kashmir [34]. Present research has found 0.9% (3) VRSA in the MRSA, comparatively higher than that shown from north part of India [35]. However, Thati et al. [36] have recently revealed 2.46% VRSA in MRSA isolates from intensive care units of tertiary care hospitals in Hyderabad. Resistance to linezolid in MRSA has not yet been reported from any part of India, but we found 0.6% (2) linezolid resistance in our isolates. The study is very important as no such work has been conducted earlier from this tribal region. We found a moderate prevalence of MRSA in Bastar, and significantly higher percentage of the isolates susceptible to the CLSI recommended panel of antibiotics. However, emergence of linezolid resistance and higher percentage of vancomycin resistance in MRSA is alarming. The probable reason for the significant variation in the antibiotic susceptibility of the isolates in our study might be due to the preferential therapeutic use of vancomycin and linezolid, the drugs of choice, as a substitute for bacterial identification and sensitivity testing in the absence of sufficient microbiology laboratory facility at this tribal region. We observed the higher generation of macrolides and fluoroquinolone more promising than the lower one. Cotrimoxazole and/or gentamycin may be considered as initial empiric treatment, but must be replaced immediately with the correct antibiotics according to the antibiogram. Although, the study highlights linezolid and vancomycin as the most sensitive agents of the entire selected panel of antibiotics, these classes must not be used commonly in therapy if the other sensitive antibiotics are available in the microbiological report. Also, it is suggestive to the respective government health authorities to pay attention to this tribal region in providing sufficient facility for microbiological diagnostics and culture sensitivity.

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Competing Interests

The authors do not have any competing interest in this manuscript.

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